

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
29 December 2004 (29.12.2004)

PCT

(10) International Publication Number
WO 2004/112851 A1

- (51) International Patent Classification⁷: **A61L 15/38**, 15/60
- (21) International Application Number:
PCT/GB2004/002696
- (22) International Filing Date: 23 June 2004 (23.06.2004)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
0314604.0 23 June 2003 (23.06.2003) GB
- (71) Applicant (for all designated States except US): **JOHN-SON & JOHNSON MEDICAL LIMITED** [GB/GB]; Airebank Mill, Gargrave, Skipton BD23 3RX (GB).
- (72) Inventor; and
- (73) Inventor/Applicant (for US only): **FOSTER, Simon** [GB/GB]; The Croft, Southampton Road, Whaddon, Salisbury, Wiltshire SP5 3DX (GB).
- (74) Agents: **JAMES, Anthony, Christopher, W., P. et al.**; Carpmaels & Ransford, 43-45 Bloomsbury Square, London WC1A 2RA (GB).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
- with international search report
 - before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: **HYDROGEL COMPOSITIONS COMPRISING ENZYMES**

(57) Abstract: A wound dressing comprising a support sheet and a layer of a synthetic hydrogel material coated onto the support sheet, wherein an active enzyme is dispersed in the synthetic hydrogel material. The hydrogel is preferably a polyacrylate or polyacrylamide, and the enzyme is preferably lactate oxidase. Also provided are methods of making such wound dressings by applying a hydrogel premix comprising a synthetic hydrogel polymer precursor and an enzyme to a solid support; followed by polymerising the premix on the support.

WO 2004/112851 A1

HYDROGEL COMPOSITIONS COMPRISING ENZYMES

Field of the Invention

The present invention relates to hydrogel compositions comprising active
5 enzymes. The invention also relates to methods of manufacture of such
compositions, and the use of such compositions in wound dressings.

Background of the Invention

Hydrogel wound dressings, in which the wound contacting layer comprises a
10 hydrogel, are advantageous because wound exudate does not generally dry and
consolidate with hydrogel. Consequently, the removal of a hydrogel dressing is
usually neither painful nor detrimental to the healing process. Hydrogel dressings
are particularly desirable for the treatment of burns. There can be difficulties in
handling the structurally weak gels in high speed manufacturing processes, and in
15 clinical practice. For this reason, the hydrogel layer may be laminated to a
support layer, for example a plastic film or a textile layer.

A number of natural, semi-synthetic, and synthetic hydrogel-forming polymers
have been proposed for use in wound dressings. The natural hydrogels include
20 gelatins, pectins, and hyaluronic acid and its derivatives. Semi-synthetic
hydrogels include modified celluloses and modified starches. Synthetic hydrogel-
forming polymers suitable for use in wound dressings include certain
polyurethanes, polyacrylates, polyacrylamides, and polyvinylpyrrolidones.
Synthetic hydrogels have the advantage that they can be prepared as a liquid
25 prepolymerisation mixture of controlled viscosity that can be applied to a suitable
substrate, and then polymerised on the substrate to provide a desired hydrogel
laminate.

For example, US-A-5160328 describes a dressing having a wound contacting
30 layer of a polyurethane hydrogel. The polyurethane gel comprises from 0% to
90% of polyhydric alcohol such as polypropylene glycol, from 6% to 60% by weight
of an isocyanate-terminated prepolymer, from 4% to 40% by weight of a
polyethylene oxide based diamine, and the balance water. The hydrogel layer is

disposed on a support layer that provides mechanical support for the relatively weak hydrogel.

EP-A-0610056 describes a hydrogel dressing comprising a wound contacting layer
5 of polyvinylpyrrolidone, which has been cross-linked by irradiation, and which is applied to a substrate. The hydrogel facing surface of the substrate comprises fibers projecting into the hydrogel. The hydrogel is cross-linked on the substrate and thereby firmly anchored thereto.

10 WO00/07638 describes polyacrylate hydrogel materials suitable for use as the wound contacting layer of wound dressings.

WO02/38097 describes wound dressings having a layer of hydrogel coated onto the wound facing layer of an apertured polymer film support sheet.

15

WO97/02811 describes a hydrogel patch for the measurement of blood glucose. The glucose drawn into the patch undergoes a reaction with a glucose oxidase in the gel, and the hydrogen peroxide thereby released is used for electrochemical determination of the glucose concentration. The preferred gel is a cross-linked
20 polyethylene oxide. The cross-linking can be carried out with a bis acrylamide in the presence of the glucose oxidase.

US-A-5648252 describes incorporating enzymes into insoluble hydrogels formed by combining an anionic hydrogel with a cationic hydrogel.

25

WO00/78332 describes incorporating fish serine proteases into medicinal compositions, including gels.

Summary of the Invention

30 It has now been found that enzymes can be included in the polymerisation mixture used to prepare synthetic hydrogels, and that surprisingly high enzyme activity is retained by the enzyme entrapped in the hydrogel after polymerisation.

Accordingly, in a first aspect the present invention provides a wound dressing comprising a synthetic hydrogel material, wherein an active enzyme is dispersed in the synthetic hydrogel material.

- 5 Typically the wound dressing of the present invention further comprises a support and the synthetic hydrogel material containing the dispersed active enzyme is coated onto the support.

- In a second aspect, the present invention provides a method of making a wound dressing comprising the steps of: preparing a hydrogel premix comprising a synthetic hydrogel polymer precursor and an enzyme; followed by polymerising the premix to produce a synthetic hydrogel material having an active enzyme dispersed therein.

- 15 Typically, the method further comprises applying a layer of the premix to a solid support; and the step of polymerising the premix is carried out on the support to produce a layer of the synthetic hydrogel material containing an active enzyme on the support.

- 20 The present invention further provides the use of a wound dressing according to the present invention for the preparation of a dressing for application to a wound.

- In a further aspect, the present invention provides a method of treatment of a wound comprising the step of applying a wound dressing in accordance with the present invention to the surface of the wound

Detailed Description of the Invention

The Support

- 30 The support may be any structure having stiffness or tensile strength greater than that of the hydrogel layer. Normally, the support is in the form of a sheet material, usually a flat sheet material. Usually, the support is made from a material that is not water soluble or water swellable. The support sheet may be formed from a

thermoplastic film-forming polymer. Preferably, the polymer is conformable but not substantially elastomeric. Suitable polymers include, but are not limited to, polyolefins such as polyethylene and polypropylene; polyesters; polyamides such as nylons; fluoropolymers such as polyvinylidene fluoride (PVDF) or
5 polytetrafluoroethylene (PTFE); olefin copolymers such as ethylene vinyl alcohol (EVA), and mixtures and laminates thereof. Preferably, the film has a thickness by weight (ASTM E252-84) of from 10 to 200 micrometers, more preferably from 25 to 100 micrometers. The support may be continuous or interrupted. For example, the support sheet may be a polymer film, which may be apertured to
10 provide liquid permeability. In other embodiments, the support sheet may comprise, or consist essentially of, a woven, nonwoven or knitted fabric.

In certain embodiments, the support sheet is adapted to block or restrict passage of liquid from a back surface to a wound facing surface of the support sheet. That
15 is to say, the support sheet allows fluid to pass through the support sheet from the wound site, but blocks or restricts flow of the fluid back through the support sheet onto the wound (also known as wet-back). Such non-wetting support sheets may for example be made from porous non-woven fabrics comprising a layer of hydrophobic fibers, or having a hydrophobic finish applied to at least the outer
20 surface thereof. Preferably, the support sheet has greater liquid permeability to the flow of liquid away from the wound facing surface than to the flow of liquid towards the wound facing surface.

In certain embodiments of this type, the support sheet is formed from a
25 substantially liquid-impermeable sheet material provided with tapered capillaries, each capillary having a base substantially in the plane of the wound facing surface of the support sheet and an apical opening remote from the wound facing surface of the support sheet and preferably in contact with the hydrogel and/or the absorbent layer. The conical capillaries provide rapid one-way wicking of fluid
30 from the front of the support sheet, with minimal wet-back. Support sheets of this type are described in GB-A-1526778, the entire content of which is incorporated herein by reference. Support sheets of this type may be manufactured, for example, by embossing or vacuum perforation of a liquid-impermeable

thermoplastic film. Preferably, the density of the capillaries is from 10 to 400 per cm², more preferably from 50 to 200 per cm². Preferably, the open area of the support sheet is from 5 to 50% of the total area, more preferably from 10 to 25% of the total area.

5

The Enzymes

The term "enzyme" refers to a polypeptide having catalytic effect in a metabolic reaction. It encompasses both therapeutic enzymes and diagnostic enzymes, but it does not encompass growth factors.

10

Suitable enzymes may be selected from the group consisting of:

- (a) antimicrobial enzymes such as lysozyme;
 - (b) oxidase enzymes such as lactate oxidase, glucose oxidase, hexose oxidase, cholesterol oxidase, galactose oxidase, pyranose oxidase, choline oxidase,
 - 15 pyruvate oxidase, oxalate oxidase, glycollate oxidase and D-aminoacid oxidases;
 - (c) catalase;
 - (d) peroxidase enzymes such as lactoperoxidase, horseradish peroxidase, iodide peroxidase, chloride peroxidase and myeloperoxidase;
 - (e) Matrix forming and degrading enzymes, including proteinases and proteases,
 - 20 for example Streptokinase, collagenase and streptodornase, bromelain, plasmin and trypsin, Urokinase, plasmin, brinolase, tissue plasminogen activator, Factor XIIIa, thrombin, Von Willibrand factor,
 - (f) Metabolic enzymes: for example Hexokinase, Phosphoglucose isomerase, phosphofructokinase, Aldose, Triose, phosphate isomerase, glyceraldehydes 3-
 - 25 phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerol mutase, enolase, pyruvate kinase, Citrate synthase, Aconitase, Isocitrate lyase, malate synthase, malate dehydrogenase;
 - (g) Lysyl oxidases; and mixtures thereof.
- 30 In especially suitable embodiments, the enzymes comprise lactate oxidase, for the following reasons.

Oxygen is a prerequisite for the formation of chemical energy within living cells. When a wound tissue becomes hypoxic, the tissue will preferentially use the glycolytic pathway to generate energy in the form of adenosine triphosphate (ATP), since the amount of oxygen is limiting.

5

Pyruvate is converted to lactate by lactate dehydrogenase, in the process generating two molecules of ATP per molecule of pyruvate hydrolysed. However, only a small fraction of the potential energy content of glucose is released by anaerobic conversion into lactate; much more energy can be released by the
10 oxidative decarboxylation of pyruvate via the citric acid cycle.

Lactate is in effect a metabolic "dead-end" in the mammalian body, as it must be converted back into pyruvate before it can be metabolised. In mammals, this reaction is only performed in the liver. Consequently, in wounds, lactate
15 concentrations often rise to levels which are detrimental to the healing process. In particular, the presence of large amount of lactic acid in the wound causes a severe drop in the pH of the wound and thus slows down the healing process; (the ideal pH for the healing process to take place in the wound is thought to be around 6.0). In addition, high levels of lactic acid upset the redox balance of the wound,
20 and impair metabolic balance in other ways.

The activity of the dressings can be specified in terms of activity units per gram of the dressing. One unit will remove 1.0 μmol of L-lactate per minute at pH6.5 at 37°C. Thus, for example, one unit of lactate oxidase activity is the amount needed
25 to oxidize 1.0 μmol of L-lactate to pyruvate and H_2O_2 per minute at pH6.5 at 37°C. Preferably, the activity (e.g. lactate oxidase activity) of the dressings is from about 0.001 units/g to about 100 units/g, more preferably from about 0.01 units/g to about 10 units/g, and most preferably from about 0.1 units/g to about 1 unit/g.

30 Most preferably, the compound or reagent comprises a lactate oxidase enzyme. Lactate oxidase may be derived from any organism or may be partially or wholly synthetic. Suitable lactate oxidase species are present in both prokaryotes and eukaryotes. From the point of view of expense, prokaryote-derived enzymes will

be preferred, although eukaryote enzymes, preferably mammalian or human, are less likely to cause immunogenic reactions in the wound site. Human lactate oxidase is most preferable.

5 The activity of pure freeze-dried lactate oxidase is about 20 to 40 units/mg. Preferably, each gram of the dressings according to the present invention contains from about 0.1mg to about 1mg of lactate oxidase, more preferably from about 1ng to about 100ng of lactate oxidase.

10 Lactate oxidase enzyme that have been engineered to possess advantageous properties over the wild type species may also be used according to the present invention. In particular, enzymes may be modified by site-directed mutagenesis to accelerate the rate at which they metabolise lactate or to reduce the immunogenicity of the protein.

15

Lactate oxidase acts to catalytically convert lactic acid into pyruvic acid that will diffuse into the environment of the wound, where it may be utilised as an energy source by the cells of the wound through its oxidative carboxylation as part of the citric acid cycle. The availability of this extra energy source will allow the cells of
20 the wound to grow more quickly. Furthermore, the pH of the wound environment will increase as the lactic acid concentration in the wound falls.

The oxygen needed for lactate oxidase reaction comes from the environment of the wound and from the atmosphere itself. The hydrogen peroxide generated as a
25 by-product of this reaction of lactate oxidase with oxygen may spontaneously decompose to release oxygen back into the wound.

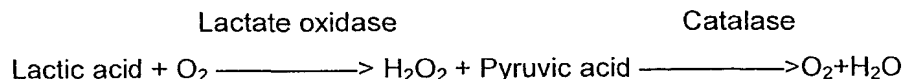
The hydrogen peroxide may also be beneficial to the wound healing process. For example, hydrogen peroxide is a bactericidal agent, acting to inhibit the growth of
30 microbes on the wound surface, thereby minimising the risk of development of clinical infections in the wound. As a by-product of this effect, this chemical acts to minimise the build-up of chemical odours developing from microbial growth in the wound.

In use, the higher the lactate concentration in the wound, the greater the activity of lactate oxidase in the wound dressing or implant that will result. Consequently, the system is self-regulating.

5

Additional compounds may also be coupled in particular to the oxidase enzymes used in of the present invention. For example, a compound can be used that accelerates the reduction of H_2O_2 into H_2O and molecular oxygen. For example, a suitable enzyme that catalyses this process is the catalase enzyme. The reaction

10 in the presence of lactate oxidase and catalase is set out below.



15 The use of catalase as a coupled enzyme has the advantage that local oxygen levels in the wound environment may be boosted, causing a concomitant increase in growth of cells in the environment of the wound. Catalase enzyme may be obtained from any source, as discussed above for lactate oxidase. Potato homogenate is a particularly good source of catalase. Catalase activity is
20 generally defined such that one unit will decompose $1.0\mu\text{mol}$ of H_2O_2 per minute at pH 7.0 at 25°C , while the H_2O_2 concentration falls from 10.3 to 9.2mM. Preferably, the catalase activity per gram of the wound dressings of the present invention is within one of the preferred ranges specified above for the lactate oxidase activity. The activity of commercially available catalase varies from about 1000 units/mg to
25 about 50,000 units/mg. It follows that the amount of catalase used to make the wound dressings of the invention is preferably about 0.01ng to about 10ng/gram of the hydrogel dry weight.

Indicator systems that are responsive to the concentration of various substances
30 such as hydrogen peroxide in a wound may also be associated with the wound dressing or implant of the present invention. For example, the indicated concentration of H_2O_2 produced by the reaction between lactate oxidase and lactic acid gives an indication of the concentration of lactate initially present in the wound

environment. This will give a physician useful information about the metabolic condition of the wound, for example an indication of the degree of hypoxia.

The indicator systems may comprise a redox indicator compound, which is usually
5 activated by a peroxidase enzyme in the presence of hydrogen peroxide.

Preferably, the indicator compound is a chromogenic compound. Suitable chromogenic substrates suitable as coupled indicators of lactate concentration include the following, along with the colour produced upon oxidation by H_2O_2 .
10 ABTS (2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) [green]; OPD (o-phenylenediamine) [orange]; TMB (3,3'-5,5'-tetramethylbenzidine) [blue]; O-dianisidine [orange]; 5AS (5-aminosalicylic acid) [brown]; DAB (3,3'-diaminobenzidine) [brown]; AEC (3-amino-9-ethylcarbazole) [blue]; 4C1N (4-chloro-1-naphthol) [blue]. All of these indicator compounds are available from
15 Sigma Chemical Company.

For most of the above indicator compounds, a means of oxidation of the compound must also be present in the dressing or implant. Any means of oxidation may be used that can be coupled stoichiometrically to the amount of
20 hydrogen peroxide present in the wound. For example, a peroxidase enzyme may be incorporated into the device, so causing the oxidation of an indicator compound. This reaction is shown below:



Preferably, the means of oxidation of the indicator compound comprises a peroxidase enzyme, more preferably horseradish peroxidase. Suitable concentrations of peroxidase enzyme and indicator can readily be determined by
30 the person skilled in the art.

The Hydrogel

The term "hydrogel layer" refers to thin, two-dimensional layer, preferably consisting essentially of the hydrogel composition. It may be a substantially unitary layer, and it may have substantially constant thickness. In certain
5 embodiments, the hydrogel layer is coextensive with the support. The advantages of the invention can be achieved with a thin hydrogel layer, which minimises the cost of the dressing. The hydrogel layer typically has a dry basis weight of from about 1 to about 2000g/m², for example about 5 to about 1000 g/m², or from 10 about to about 500g/m², or from about 10 to about 200g/m², or
10 from about 10 to about 100g/m², or from 10 about to about 50g/m².

The hydrogel layer may be continuous or discontinuous. Continuous hydrogel layers extend over and cover any apertures in the support. Such continuous layers provide the advantage of temporarily blocking (gel blocking) the passage of
15 wound fluid through the support until the hydrogel is saturated. This helps to prevent drying out of wounds having low rates of exudate production.

In other embodiments the hydrogel layer is apertured. In some embodiments it is apertured in register with apertures in the support so as not to obstruct passage of
20 fluid through the support even when the hydrogel is fully swelled. In these embodiments, there is substantially no hydrogel initially present in or covering the apertures of the support.

The term "hydrogel" refers generally to materials that interact with wound fluid
25 under physiological conditions to form a hydrated gel at the wound surface. That is to say, the hydrogel polymer forms a gel with water under physiological conditions of temperature and pH. Such hydrogel layers are formed by medically acceptable synthetic macromolecular materials that have the ability to swell and absorb fluid while maintaining a strong integral structure. The hydrogel material is
30 normally substantially insoluble in water under physiological conditions, whereby the hydrogel is not washed away by the wound fluid. The hydrogel may be may be bioabsorbable. That is to say, it may undergo full degradation and resorption *in vivo* in the mammalian body.

A suitable composition for the hydrogel layers of the present invention comprises a plasticised three-dimensional matrix of cross-linked polymer molecules. Such polymers can have sufficient structural integrity to be self-supporting even at very
5 high levels of internal water content, with sufficient flexibility to conform to the surface contours of the human skin.

Exemplary insoluble gels include polyurethane gels, certain cross-linked polyacrylate gels, and gels formed by polymerizing vinyl alcohols, vinyl esters,
10 vinyl ethers, N-vinyl pyrrolidone, PLURONIC (Registered Trade Mark) (block polyethylene glycol, block polypropylene glycol) polystyrene-, maleic acid, and mixtures thereof. The term "polyurethane" herein refers to a polymer having a plurality (preferably at least 10, 20 or 100) urethane and/or urea linkages in the main polymer chain. The term "polyacrylate" herein refers to a polymer wherein
15 the main polymer chain is formed by polymerising or copolymerising one or more carboxy vinyl monomers (acrylates or acrylate derivatives), for example one or more monomers selected from the group consisting of meth(acrylic) acid, acrylamide, N-substituted acrylamides, acylamidopropane sulphonic acid, NN-dimethylacrylamide diacetone acrylamide, acryloyl morpholine, and mixtures
20 thereof.

Preferably, the gel adheres strongly to the surface of the support material to resist washing off by wound fluid. In certain embodiments the gel may be directly coated or chemically covalently bonded to the support material.

25

Suitably, the hydrogel layer comprises a hydrogel material selected from polyurethane gels, and polyacrylates, i.e. polymers of acrylates and acrylate derivatives such as modified acrylamides and mixtures thereof. Suitably, the gel-forming polymer of the hydrogel layer consists essentially of polyurethane or
30 polyacrylate. In certain embodiments, the hydrogel layer comprises a hydrogel material of the kind described in WO00/07638 or WO00/065143, the entire contents of which are incorporated herein by reference.

Suitably, the gels are cross-linked, and the cross-linking may be either covalent or ionic. In certain embodiments, the hydrogel material further comprises from 5 to 50% by weight on a dry weight basis of one or more humectants such as glycerol.

- 5 The hydrogel composition used in the present invention typically consists essentially of a cross-linked hydrophilic polymer of a hydrophilic monomer and optionally one or more comonomers, together with water and/or one or more organic plasticisers, and optionally together with one or more additives selected from surfactants, polymers, pH regulators, bioactive compounds and mixtures
10 thereof, with less than about 10% by weight of other additives.

In certain embodiments, the hydrophilic acrylate monomer may be one or more ionic monomers. The hydrophilic monomer may be a mixture of anionic and cationic monomers, typically in substantially equimolar amounts. The one or more
15 ionic monomers may for example be selected from: 2-acrylamido-2-methylpropane sulphonic acid or an analog thereof or one of its salts (e.g. an ammonium or alkali metal salt such as a sodium, potassium or lithium salts); acrylic acid or an analogue thereof or one of its salts (e.g. an alkali metal salt such as a sodium, potassium or lithium salt); and/or a polymerisable sulphonate or a salt thereof (e.g.
20 an alkali metal salt such as a sodium, potassium or lithium salt), more particularly acrylic acid (3-sulphopropyl) ester or an analogue thereof, or a salt thereof. The term "analogue" in this context refers particularly to substituted derivatives of 2-acrylamido-2-methylpropane sulphonic acid, of acrylic acid or of acrylic acid (3-sulphopropyl) ester.

25

A particularly suitable ionic monomer is a sodium salt of 2-acrylamido-2-methylpropane sulphonic acid, commonly known as NaAMPS and/or acrylic acid (3-sulphopropyl) ester potassium salt, commonly known as SPA or SPAK.

- 30 The hydrophilic acrylate monomer may alternatively or additionally be non-ionic, for example it may be selected from acrylamide or a mono- or di-N-alkylacrylamide or an analog thereof. The term "analog" in this in this context refers to non-ionic water soluble monomers containing an alkyl or substituted alkyl group linked to a

carbon-carbon double bond via an amido or alkylamido (-CO.NH- or -CO.NR-) function. Examples of such analogs include diacetone acrylamide (N-1,1-dimethyl-3-oxobutyl-acrylamide), vinyl lactams, N-alkylated acrylamides, N,N-dialkylated acrylamides, N-vinyl pyrrolidone, N-acryloyl morpholine and any
5 mixture thereof, particularly N-acryloyl morpholine.

Conventional cross-linking agents are suitably used to provide the necessary mechanical stability and insolubility to the hydrogel. The amount of cross-linking agent required will suitably from about 0.01% to about 0.5%, particularly from
10 about 0.05% to about 0.4%, most particularly from about 0.08% to about 0.3%, by weight of the total polymerisation reaction mixture. Typical cross-linkers for use in cross-linking of polyacrylates useful in the present invention include tripropylene glycol diacrylate, ethylene glycol dimethacrylate, triacrylate, polyethylene glycol diacrylate (polyethylene glycol (PEG) molecular weight between about 100 and
15 about 4000, for example PEG400 or PEG600), and methylene bis acrylamide.

The one or more organic plasticisers, when present, may suitably comprise any of the following either alone or in combination: at least one polyhydric alcohol (such as glycerol, polyethylene glycol, or sorbitol), at least one ester derived therefrom,
20 at least one polymeric alcohol (such as polyethylene oxide) and/or at least one mono- or poly-alkylated derivative of a polyhydric or polymeric alcohol (such as alkylated polyethylene glycol). Glycerol is the preferred plasticiser. An alternative preferred plasticiser is the ester derived from boric acid and glycerol. When present, the organic plasticiser may comprise up to about 45% by weight of the
25 hydrogel composition.

The hydrogel compositions may comprise Non-ionic Surfactants, Anionic Surfactants, Cationic Surfactants, or mixtures thereof. The total amount of surfactant, if present, is suitably up to about 10% by weight of the hydrogel
30 composition, preferably from about 0.05% to about 4% by weight.

In other embodiments, the hydrogel may be a polyurethane gel, for example a gel formed by reaction of an isocyanate prepolymer with a chain extending compound

such as a diamine or water. For example, the hydrogel material may be formed from an aqueous mixture including from about 0% to about 90% by weight polyhydric alcohol; from about 6% to about 60% by weight aliphatic diisocyanate-terminated prepolymer; from about 4% to about 40% by weight polyethylene oxide-based polyamine; up to about 2% by weight sodium chloride; and the balance water. A more suitable hydrogel composition for forming the polyurethane hydrogel layer comprises from about 15% to about 30% by weight of a polyhydric alcohol selected from a group consisting of polypropylene glycol, polyethylene glycol and glycerine, from about 8% to about 14% by weight isophorone-diisocyanate-terminated prepolymer, from about 5% to about 10% by weight polyethylene oxide-based diamine, up to about 1% by weight of a salt, and the remaining percentage water. Suitable isocyanate capped prepolymers for the production of polyurethane hydrogels, and methods of manufacture thereof are described in EP-A-0335669 and WO9902587, the entire contents of which are incorporated herein by reference.

The hydrogel composition used in the present invention may include one or more additional ingredients, which may be added to the pre-polymerisation mixture or the polymerised product. Suitable additional ingredients are selected from the group consisting of water, organic plasticisers, surfactants, polymers, pH regulators, colorants and mixtures thereof. The polymers can for example be biopolymers, such as gelatin, pectin or hyaluronic acid.

In the wound dressings according to the present invention, the enzyme may be covalently bonded to the polymer chain of the synthetic hydrogel material. The enzyme is frequently partially or completely copolymerized with the synthetic hydrogel material.

The hydrogel layer may be provided on the wound facing surface of the wound dressing according to the present invention. The hydrogel then provides a wound friendly, soft, non-adherent and therapeutic wound contacting surface to the dressing. In alternative embodiments, the hydrogel layer is provided on the back surface of the support sheet, opposite the wound facing surface. The provision of

a hydrogel layer adjacent to the back surface of such a support sheet enables a moist wound environment to be maintained for prolonged periods, over a wide range of wound exudation rates. In use, the support sheet continues to wick away wound fluid to prevent excessive moisture in the wound. When the rate of wound exudate production falls, the hydrogel absorbs moisture vapor from the absorbent layer and preserves a moist wound surface. The hydrogel does not give rise to substantially increased wet-back through the support sheet. Loss of hydrogel through the apertures of the support sheet is minimal, and the hydrogel does not interfere with the absorbent layer.

10

Other Dressing Components

The dressing may further comprise a backing layer over the back face of the support sheet. The backing layer supports the support sheet and any optional intermediate absorbent layer and suitably provides a barrier to passage of microorganisms through the dressing. The backing layer may extend beyond at least one edge of the hydrogel layer to provide an adhesive-coated margin adjacent to the said edge for adhering the dressing to a surface, such as to the skin of a patient adjacent to the wound being treated. An adhesive-coated margin may extend around all sides of the hydrogel layer, so that the dressing is a so-called island dressing. However, it is not necessary for there to be any adhesive-coated margin.

The backing layer is normally substantially liquid-impermeable. The backing sheet is preferably semipermeable. That is to say, the backing sheet is preferably permeable to water vapour, but not permeable to liquid water or wound exudate. Preferably, the backing sheet is also microorganism-impermeable. Suitable continuous conformable backing sheets will preferably have a moisture vapor transmission rate (MVTR) of the backing sheet alone of 300 to 5000 g/m²/24hrs, preferably 500 to 2000 g/m²/24hrs at 37.5°C at 100% to 10% relative humidity difference. The backing sheet thickness is preferably in the range of 10 to 1000 micrometers, more preferably 100 to 500 micrometers.

The MVTR of the dressing according to the present invention as a whole is lower than that of the backing sheet alone, because the hydrogel layer and support sheet partially obstruct moisture transfer through the dressing. Preferably, the MVTR of the dressing (measured across the island portion of the dressing) is from 5 20% to 80% of the MVTR of the backing sheet alone, more preferably from 20% to 60% thereof, and most preferably about 40% thereof. It has been found that such moisture vapor transmission rates allow the wound under the dressing to heal under moist conditions without causing the skin surrounding the wound to macerate.

10

Suitable polymers for forming the backing sheet include polyurethanes and polyalkoxyalkyl acrylates and methacrylates such as those disclosed in GB-A-1280631. Preferably, the backing sheet comprises a continuous layer of a high density blocked polyurethane foam that is predominantly closed-cell. A suitable 15 backing sheet material is the polyurethane film available under the Registered Trade Mark ESTANE 5714F.

The adhesive (where present) layer should be moisture vapor transmitting and/or patterned to allow passage of water vapor therethrough. The adhesive layer is 20 preferably a continuous moisture vapor transmitting, pressure-sensitive adhesive layer of the type conventionally used for island-type wound dressings, for example, a pressure sensitive adhesive based on acrylate ester copolymers, polyvinyl ethyl ether and polyurethane as described for example in GB-A-1280631. The basis weight of the adhesive layer is preferably 20 to 250 g/m², and more preferably 50 25 to 150 g/m². Polyurethane-based pressure sensitive adhesives are preferred.

The area of the optional absorbent layer is typically in the range of from 1cm² to 200cm², more preferably from 4cm² to 100cm².

30 The optional absorbent layer may be any of the layers conventionally used for absorbing wound fluids, serum or blood in the wound healing art, including foams, sponges, gauzes, and nonwoven fabrics, and combinations thereof. Superabsorbents or hydrogels may be dispersed in the optional absorbent layer

to improve liquid absorbency and retention. Preferably, the absorbent layer comprises a layer of absorbent foam, such as an open celled hydrophilic polyurethane foam prepared in accordance with EP-A-0541391, the entire content of which is expressly incorporated herein by reference. In other embodiments, the
5 absorbent layer may be a nonwoven fibrous web, for example a carded web of viscose staple fibers. The basis weight of the absorbent layer may be in the range of 50-500g/m², such as 100-400g/m². The uncompressed thickness of the absorbent layer may be in the range of from 0.5mm to 10mm, such as 1mm to 4mm. The free (uncompressed) liquid absorbency measured for physiological
10 saline may be in the range of 5 to 30 g/g at 25°C.

The optional absorbent layer is a separate layer from the hydrogel layer in the wound dressings according to the present invention. In certain embodiments the hydrogel layer and the optional absorbent layer are adjacent.

15

Preferably, the multilayer wound dressing according to the invention further comprises one or more protective cover sheets over the support sheet and any exposed hydrogel and/or adhesive. For example, these may comprise one or more release-coated paper cover sheets. Preferably, the dressing according to
20 the present invention is sterile and packaged in a microorganism-impermeable container.

The wound dressing or implant according to the present invention may also contain a medicament, in addition to the enzyme or enzymes entrapped in the
25 hydrogel. Suitable medicaments will be well known to those of skill in the art and include antiseptics, such as povidone iodine or silver sulfadiazine; antibiotics such as enthomycin, neomycin, bacitracin, gentamycin, framycetin, thyrotrycin, polymyxin B, gramicidin, fusidic acid, chloramphenicol, tetracycline and its derivatives, minocycline chlortetracycline, hydrochloride, meclocyclin, penicillin
30 and its derivatives, ampicillin or a cephalosporin; steroidal anti-inflammatories such as hydrocortisone, betamethasone, dexamethasone, prednisolone, and their derivatives; non- steroidal anti-inflammatories such as indomethacin, ketoprofen, ibuprofen and diclofenac; anaesthetics such as cocaine, benzocaine, procaine or

lignocaine; analgesics such as aspirin; and anti-oxidants such as Vitamin E, Vitamin C, Zinc, selenium or cysteine. The medicaments may be present in the hydrogel layer, and/or medicaments may be present in any of the other layers of the dressing.

5

Method of Manufacture

The method of making a wound dressing according to the present invention comprises the steps of: preparing a hydrogel premix comprising a synthetic hydrogel polymer precursor and an enzyme; applying a layer of the premix to a solid support; followed by polymerising the premix on the support to produce a layer of synthetic hydrogel material on the support, wherein an active enzyme is dispersed in the synthetic hydrogel material.

15 In certain embodiments, the hydrogel premix is UV-curable and the step of polymerizing comprises curing the hydrogel with ultraviolet light. These embodiments include the hydrogels based on ionic acrylate and acrylamide-derived monomers described in detail above. Suitable catalysts for the polymerisation may also be present.

20 In other embodiments, the hydrogel premix comprises an isocyanate-capped prepolymer, and said step of polymerising comprises allowing said prepolymer to react with a chain extending compound. Suitable chain extending compounds include water, diols and polyols, and diamines and polyamines. Suitable catalysts for the polymerisation may also be present.

25

The hydrogel premix layer may be applied by spraying or, preferably, by a printing or transfer process. Apertures may be formed in the hydrogel layer for example by drilling with a hollow needle. In other embodiments, the apertures may be formed by casting the support sheet in a mold having an array of projections corresponding to the apertures, followed by peeling the hydrogel layer from the mold and applying it in register to the support sheet. In yet other embodiments, the hydrogel layer may be applied to an apertured substrate by the method of WO00/65143, the entire content of which is incorporated herein by reference.

Specific embodiments of the present invention will now be described further, by way of example.

5 Example 1

A hydrogel layer for a dressing according to the present invention is prepared from the following materials:

3-sulphylacrylate potassium salt (SPA) (Sigma Chemical Company)

1-hydroxy-cyclo-hexyl-phenyl-ketone (Sigma Chemical Company)

10 Polyethylene glycol diacrylate (Sigma Chemical Company)

Lactate oxidase (Sigma Chemical Company)

Phosphate buffered Saline (PBS) (GIBCO CO.)

The lactate oxidase was diluted in PBS with a dilution of 4 units/ml. This solution
15 was stored at -20°C and defrosted when required.

50g of SPA were weighed into dark glassware. To this 50ml of double distilled water were added. This was mixed until all the powder had dissolved and was stored at 4°C.

20

6g of 1-hydroxy-cyclo-hexyl-phenyl-ketone were weighed into dark glassware. 20ml of polyethylene glycol diacrylate were then added and mixed until all the powder had dissolved. This was again stored at 4°C.

25 To produce the hydrogel sheets with the enzyme in the gel, 4ml of 50% SPA solution were taken and pipetted into a mixing tube. Then 15µl of cross-linker solution and 40ml of the lactate oxidase solution were mixed with the SPA solution. The mixture was then placed on the UV exposure unit and irradiated for thirty seconds, whilst polymerisation took place. The hydrogel formed was then
30 stored at 4°C until it was needed for testing.

Procedure 1

The activity of the enzyme-loaded hydrogels was evaluated by means of Hydrogen Peroxide Generation Assays

5 Hydrogen peroxide (H_2O_2) generation assays utilise the reaction of H_2O_2 with ortho-phenylene diamine (OPDA) in the presence of horseradish peroxidase. The reaction mixture is initially colourless, but develops a yellow colouring with the presence of H_2O_2 . It was the development of this colour that was measured quantitatively using the UV spectrophotometer.

10

The method used trans-well plates with the hydrogel placed within the well; this was then lowered into a solution containing both the lactate and the assay solutions. This trans-well plates suspend the hydrogel in the solution above a membrane, which allows the free passage of lactate into, and hydrogen peroxide
15 out of, the hydrogel, along with the assay solutions. This means that the assay indicated the generation of hydrogen peroxide by enzyme entrapped within the hydrogel, as well as by any enzyme that diffused into the solution.

The method also included three controls, one positive and two negative. The
20 negative controls were: a) the hydrogel, containing no lactate oxidase, with the lactate and assay solutions, and b) the lactate and assay solutions on their own. The positive control used was the lactate and assay solution, but this time in conjunction with a sample of the lactate oxidase. The lactate oxidase solution used was of equal mass and dilution to that of the lactate oxidase found in the
25 hydrogel to allow direct comparison.

The test method comprised the following steps:

1. First the trans-well inserts were removed from the plate. This was done so
30 that the hydrogels could be placed into them without coming into contact with the lactate or assay solutions. By doing this all the wells could be put into contact with the solutions at the same time, giving each well as close to the same start time as

possible. This was important for accuracy, especially when shorter time points were being measured.

2. Then 6mm biopsy punches were taken from the hydrogels being tested,
5 including the control hydrogel, and placed onto their relevant trans-well membranes.
3. 0.333ml of 20mM lactate solution were added to each well followed by 0.333ml of HRP and finally 0.333ml of OPDA solution.
- 10 4. The trans-well inserts were then returned to their appropriate wells, and 2 units/ml lactate-oxidase control solutions was pipetted into its wells.
5. Immediately the samples were covered in aluminum foil and placed into the
15 incubator.
6. Time point readings were taken using the UV spectrophotometer every half an hour, for the first two hours, and then every hour for the next three hours.
- 20 The standard curves were plotted with absorbency against concentration of hydrogen peroxide and the equation for the line was calculated. The results for each of the solutions were taken and the absorbency values were converted to concentration of hydrogen peroxide using the standard curve. These concentrations were then taken and the mean value for each solution was
25 calculated for each time point. A photograph was also taken to show where hydrogen peroxide had been produced within the hydrogel.

The results for the liquid bath showed the positive control behaving as would be expected. The hydrogel with the enzyme added before polymerisation did not
30 develop any color in the supernatant, indicating that no active enzyme was leaching from the hydrogel into the liquid bath. In other words, the active enzyme was substantially entirely entrapped within the hydrogel.

It could be seen from the development of yellow colour within the hydrogel that lactate was being converted into pyruvate within the hydrogel. By looking at the photograph and comparing to the standard curve, it could be seen that the enzyme within the hydrogel kept most of its activity during the process of polymerisation.

- 5 This comparison, although subjective, does give a reasonable indication that the hydrogen peroxide produced within the hydrogel is of similar concentration to that of the positive control at the end of the experiment. This would only be expected if the majority of the enzyme activity had survived the process of polymerisation. This was quite surprising considering the harsh conditions to which the lactate-
- 10 oxidase was exposed during polymerisation,

The above embodiment has been described by way of example only. Many other embodiments falling within the scope of the accompanying claims will be apparent to the skilled reader.

CLAIMS

1. A wound dressing comprising a support sheet and a layer of a synthetic hydrogel material coated onto the support sheet, wherein an active enzyme is
5 dispersed in the synthetic hydrogel material.
2. A wound dressing according to claim 1, wherein the support sheet comprises a polymer film or a woven, nonwoven or knitted fabric sheet.
- 10 3. A wound dressing according to any preceding claim, wherein the support sheet is adapted to block or restrict passage of liquid from a back surface of the sheet to a wound facing surface of the sheet.
4. A wound dressing according to any preceding claim, wherein the hydrogel
15 layer has a dry basis weight of from 10 to 200g/m².
5. A wound dressing according to claim 1 or 2, wherein the synthetic hydrogel material is cross-linked hydrophilic polymer of a hydrophilic monomer and optionally one or more comonomers, together with water and/or one or more
20 organic plasticisers, and optionally together with less than about 10% of one or more additives selected from surfactants, polymers, pH regulators, bioactive compounds and mixtures thereof.
6. A wound dressing according to any preceding claim, wherein the hydrogel
25 layer comprises a hydrogel material selected from gels formed from vinyl alcohols, vinyl esters, vinyl ethers and carboxy vinyl monomers, meth(acrylic) acid, acrylamide, N-vinyl pyrrolidone, acylamidopropane sulphonic acid, pluronic (block polyethylene glycol, block polypropylene glycol)polystyrene maleic acid, NN-dimethylacrylamide, diacetone acrylamide or acryloyl morpholine.
30
7. A wound dressing according to any preceding claim, wherein the hydrogel layer comprises a plasticiser.

8. A wound dressing according to any preceding claim, wherein the hydrogel layer is apertured in register with apertures in the support layer.
9. A wound dressing according to any preceding claim, wherein the enzyme is
5 chemically bonded with the synthetic hydrogel material.
10. A wound dressing according to any preceding claim, wherein the enzyme is selected from the group consisting of:
- (a) antimicrobial enzymes such as lysozyme;
 - 10 (b) oxidase enzymes such as lactate oxidase, glucose oxidase, hexose oxidase, cholesterol oxidase, galactose oxidase, pyranose oxidase, choline oxidase, pyruvate oxidase, oxalate oxidase, glycollate oxidase and D-aminoacid oxidases;
 - (c) catalase;
 - (d) peroxidase enzymes such as lactoperoxidase, horseradish peroxidase, iodide
15 peroxidase, chloride peroxidase and myeloperoxidase;
 - (e) Matrix forming and degrading enzymes, including proteinases and proteases, for example Streptokinase, collagenase and streptodornase, bromelain, plasmin and trypsin, Urokinase, plasmin, brinolase, tissue plasminogen activator, Factor XIIIa, thrombin, Von Willibrand factor,
 - 20 (f) Metabolic enzymes: for example Hexokinase, Phosphoglucose isomerase, phosphofructokinase, Aldose, Triose, phosphate isomerase, glyceraldehydes 3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerol mutase, enolase, pyruvate kinase, Citrate synthase, Aconitase, Isocitrate lyase, malate synthase, malate dehydrogenase;
 - 25 (g) Lysyl oxidases; and mixtures thereof.
11. A wound dressing according to any preceding claim, further comprising an layer of wound-fluid absorbent material.
- 30 12. A wound dressing according to any preceding claim, wherein the dressing further comprises a substantially liquid-impermeable backing layer covering the hydrogel layer and the support sheet and the optional absorbent layer.

13. A wound dressing according to claim 8, wherein the backing layer extends beyond at least one edge of the hydrogel layer and support sheet to provide an adhesive-coated margin adjacent to said edge for adhering the dressing to a surface.

5

14. A wound dressing according to any preceding claim, further comprising one or more protective cover sheets over the wound facing surface of the hydrogel layer and support sheet.

10 15. A wound dressing according to any preceding claim, wherein the dressing is sterile and packaged in a microorganism-impermeable container.

16. A method of making a wound dressing comprising the steps of:
preparing a hydrogel premix comprising a synthetic hydrogel polymer precursor and an enzyme;
15 applying a layer of the premix to a solid support; followed by polymerising the premix on the support to produce a layer of synthetic hydrogel material on the support, wherein an active enzyme is dispersed in the synthetic hydrogel material.

20

17. A method according to claim 16, wherein the hydrogel premix is UV-curable and the step of polymerizing comprises curing the hydrogel with ultraviolet light.

18. A method according to claim 16, wherein the hydrogel premix comprises an isocyanate-capped prepolymer, and said step of polymerising comprises allowing
25 said prepolymer to react with a chain extending compound.

30

INTERNATIONAL SEARCH REPORT

Application No

/GB2004/002696

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A61L15/38 A61L15/60

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61L A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 40 26 153 A (SEBAPHARMA GMBH & CO) 20 February 1992 (1992-02-20) column 1, lines 6-8 column 2, lines 10-15,26-40 column 3, lines 8-17 column 4, lines 8-28 claims	1-18
X,P	WO 03/090800 A (INSENSE LTD ; AUSTIN ANDREW JOHN (GB); DAVIS PAUL JAMES (GB)) 6 November 2003 (2003-11-06) page 3, paragraphs 2,3 page 5, paragraphs 3,4 page 6, paragraph 1 page 8, paragraphs 2,3 page 13, paragraphs 2,3 claims; figures	1-18

-/-

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

19 October 2004

Date of mailing of the international search report

27/10/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Böhm, I

INTERNATIONAL SEARCH REPORT

Application No
/GB2004/002696

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/38097 A (JOHNSON & JOHNSON MEDICAL LTD ; ADDISON DEBORAH (GB); SILCOCK DEREK WA) 16 May 2002 (2002-05-16) claims 5-9,14-17,21-23 the whole document	1-18
A	WO 00/65143 A (MUNRO HUGH SEMPLE ; FIRST WATER LTD (GB); LAWRENCE STEVEN JOHN (GB)) 2 November 2000 (2000-11-02) page 4, lines 1-6 claims	16,17
A	US 4 576 817 A (MONTGOMERY ROBERT E ET AL) 18 March 1986 (1986-03-18) column 1; claims	1
A	US 5 648 252 A (DUMITRIU SEVERIAN ET AL) 15 July 1997 (1997-07-15) abstract column 1, lines 8-14 column 2, lines 6-15	1
A	US 2002/018802 A1 (MEYER-INGOLD WOLFGANG ET AL) 14 February 2002 (2002-02-14) paragraphs '0001!, '0008! - '0010!, '0015!	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Application No

GB2004/002696

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
DE 4026153	A	20-02-1992	DE 4026153 A1	20-02-1992
			AT 114482 T	15-12-1994
			DE 59103721 D1	12-01-1995
			DK 543868 T3	15-05-1995
			WO 9203172 A1	05-03-1992
			EP 0543868 A1	02-06-1993
			ES 2064118 T3	16-01-1995
			JP 6500028 T	06-01-1994
WO 03090800	A	06-11-2003	EP 1358893 A1	05-11-2003
			WO 03090800 A1	06-11-2003
WO 0238097	A	16-05-2002	AU 1255202 A	21-05-2002
			EP 1333788 A1	13-08-2003
			WO 0238097 A1	16-05-2002
			JP 2004520096 T	08-07-2004
WO 0065143	A	02-11-2000	AT 247188 T	15-08-2003
			AU 765572 B2	25-09-2003
			AU 4417300 A	10-11-2000
			CA 2367657 A1	02-11-2000
			DE 60004511 D1	18-09-2003
			DE 60004511 T2	17-06-2004
			EP 1175527 A1	30-01-2002
			WO 0065143 A1	02-11-2000
			JP 2002542962 T	17-12-2002
			US 2003054024 A1	20-03-2003
US 4576817	A	18-03-1986	EP 0236610 A1	16-09-1987
US 5648252	A	15-07-1997	IL 109079 A	22-02-1998
			US 5858392 A	12-01-1999
US 2002018802	A1	14-02-2002	DE 19813663 A1	07-10-1999
			US 2002197257 A1	26-12-2002
			AU 764641 B2	28-08-2003
			AU 2133499 A	07-10-1999
			EP 0945144 A2	29-09-1999
			US 6156334 A	05-12-2000